PROCEEDINGS OF THE 47th. ANNUAL COCCIDIOIDOMYCOSIS STUDY GROUP MEETING

Meeting Number 47
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ABSTRACTS

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John N. Galgiani, M.D.
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Address editorial correspondence to:
Dr. John N. Galgiani, M.D.
Medical Service (MS1-111)
Southern Arizona VA Health Care System
3601 South 6th. Ave.
Tucson, AZ 85723 USA

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Chairperson: Demo Pappagianis

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ABSTRACTS

1.Integrated Epidemiological Study of Valley Fever: Update on Household Survey

M.K. O'Rourke[1], J. Tabor [1&2,]
University of Arizona: Arizona College of Public Health [1]
and College of Agriculture and Life Sciences[2].

Background: The Arizona Disease Control Research Commission awarded the University of Arizona a three-year grant to study smoking and other risk factors for symptomatic coccidioidomycosis in the greater Tucson area of Arizona. The study integrates epidemiological survey methods with environmental sampling and analysis using PCR methods to better assess exposure to the etiologic agent, Coccidioides immitis. A cross-sectional telephone survey, stratified by soil and socio-economic factors using a GIS, identified cases and potential controls for a population based case-control study of the disease that uses information from more detailed personal interviews.

Methods: A probability proportional to size household survey design was used to screen for cases and identify potential controls by telephone. Primary sampling units (102) are based on 2000 Census block groups and stratified on three soil-landscape types and two socio-economic classes. Concurrently soil samples from case residences and air samples collected from a reference site are collected, cultured in the Valley Fever Center for Excellence's BL-3 laboratory and analyzed using PCR techniques.

Results: We are half way into the three year grant. Study area block group populations have a much wider range than described by the Census Bureau and complicated the study's design and implementation. Out of 26 of the study's 102 primary sampling unit surveyed, approximately 10% of direct marketing addresses are non-deliverable and 26% of the telephone numbers are invalid. Of the households contacted approximately 59% (n=1813) have participated in the survey.

2. Ecological Study of Canine Valley Fever to Assess Environmental Antecedents of Disease

J. Tabor[1&2], M. Orbach[1], L. Shubitz[1], and M. Lebowitz[2].
University of Arizona, College of Agriculture[1]
and Life Sciences and Arizona College of Public Health[2]

Background: The endemic regions of *Coccidioides immitis* have well-known general ecologic characteristics, however little is known about the specific ecologic niche required for *C. immitis* to flourish. The CDC and Association of Teachers of Preventive Medicine awarded the University of Arizona a two-year grant to study the environmental antecedents of coccidioidomycosis using canine cases in the Phoenix and Tucson areas of Arizona that are reported by veterinarians and diagnostic laboratories.

Methods: The study used a geographical information system (GIS) to correlate resident location of cases with environmental factors. Soil samples collected near case residences were cultured in the Valley Fever Center for Excellence's BL3 laboratory and then analyzed using PCR techniques. GIS data of resident locations is much more developed and available in the Tucson area than the Phoenix area so the analysis was limited to cases in the Tucson area. Canine cases were stratified by two levels of urbanization and by eleven soil types then analyzed using a GIS.

Results: Dogs residing in periurban areas are 1.5 times more likely to have symptomatic valley fever than dogs residing in urban areas. Also dogs residing on some soil types are up to 1.9 times more likely of having symptomatic disease than dogs residing on other soil types. Laboratory isolation of *C. immitis* from soil samples however has proven elusive and prevented us from directly predicting the pathogen's occurrence.

Discussion: Soil types and levels of urbanization show significant differences in canine disease prevalence. Dogs may serve as good sentinels for human disease and risk of exposure because dogs are generally more restricted to a single location and have higher disease prevalence than people. Improved cultural and molecular methods are needed for detection of *C. immitis* in the soil and for improving disease prevention strategies.

3. Seasonal climate variability and Coccidioidomycosis in the Tucson Region

Andrew C. Comrie
University of Arizona, Tucson, Arizona

Coccidioidomycosis (Valley Fever) data from the University of Arizona Student Health Center in Tucson were examined to define the seasonal pattern of the disease in southern Arizona, and to identify significant seasonal correlations between climatic controls and disease variability. Higher coccidioidomycosis rates were found for fall (Oct-Nov) and winter (Dec-Mar), with a minor peak in the late foresummer (June). Lower rates were found in the late winter/spring (Mar-May) and during the monsoon (July-Sep). Significant correlations to climate conditions were found for all seasons, but the strongest results were for winter (Dec-Mar) and spring/foresummer (Apr-Jun). While details are complex, elevated coccidioidomycosis rates follow moister conditions 5-10 months (2-3 seasons) earlier, with warmer and drier and sometimes windier conditions 0-7 months (concurrently to 2 seasons) earlier. This work enhances knowledge of potential links between climate and the soil fungus *Coccidioides immitis*, and it improves understanding and possible

anticipation of coccidioidomycosis outbreaks.

4. Resurgence of coccidioidomycosis in Kern County, 2001-2002

Jinadu BA, Emery KW, Chaput EK, El Gamal YH, Nguyen S, Rush TH

Coccidioidomycosis, disease caused by the fungus *Coccidioides immitis*, is highly endemic in the southern San Joaquin Valley, and remains a disease of public health importance in this area. In the past two years, an increase in coccidioidomycosis incidence has been noted in Kern County, California (1995-2000 annualized incidence: 63.5/100,000; 2001-2002 annualized incidence: 144.9/100,000). To better characterize this observed increase of reported coccidioidomycosis cases, several modeling techniques were employed. Analyses of climate and demographic variables were performed, to evaluate the efficacy of a model for predicting monthly case numbers. Analysis included bivariate correlation analyses to identify variables to include in the multivariate model. A variety of wind, temperature, and precipitation variables were significantly correlated with monthly case numbers. Predictive models were developed using multivariate regression analysis to estimate incident coccidioidomycosis cases. Measures of wind velocity and airborne dust (PM10) were included in the final model as positively associated with incident cases. Number of building permits was also included in the model, with a negative association, but the authors believe that this is a coincident finding. Spatial analysis of coccidioidomycosis cases was also performed to clarify the spatial and temporal distributions during the recent resurgence. A geographic information system (GIS), census data, and incidence data were employed to describe and quantify the spatial and temporal patterns observed among coccidioidomycosis cases. Reported cases from 1999-2002 were geocoded, based on home address. Annualized incidence rates by census tract showed a spatial distribution of higher disease burden in the central and western areas of Kern County. Further investigation, using spatiotemporal cluster analysis (SaTScan), yielded significantly elevated areas of disease in 2001-2002 in certain western and central census tracts (RR=1.5, p=0.001). These results – both the climatic and spatial analyses – provide statistical support to the observations of public health and health care professionals in Kern County. By expanding our understanding of the distribution of this disease, and hopefully, developing effective predictive models, the Kern County Department of Public Health seeks to improve the health of our residents by enhancing targeted education, awareness, and prevention efforts in the county.

5. Roles of Cytokines, Chemokines, and other immunomodulating molecules (IMM) in Murine Coccidioidal Meningitis (CM): primary of TNF-alpha.

P. Kamberi,[1-3] R.A. Sobel,[4,5] J.M. Striebel,[1] K.V. Clemons,[1-3] D.A. Stevens,[1-3] and P.L. Williams[1]

California Institute for Medical Research[1] and Department of Medicine, Division of Infectious Diseases,[2] Santa Clara Medical Center, San Jose, California; Department of Medicine, Division of Infectious Diseases and Geographic Medicine,[3] Department of Pathoogy,[4] Stanford University Medical School, Stanford, California

There is accumulating evidence that NO, TNF-a, IL-1b and IL-6 play key roles as effectors in bacterial meningitis. However, little is known about the role of these molecules in other central nervous system (CNS) infections, including CM. We have previously reported the up-regulation of several IMMs (IL-6, TGF-b, iNOS,

IFN-g, IL-2, IL-10, IL-1, TNF-a and CCR-1) in the basilar artery and MMP-9 in the CSF of rabbits with CM. In the present study, mice genetically deficient for IMM were used to investigate the roles of specific IMMs in CM. Animals (10 mice per experimental group) were infected with ~70 cfu of *C. immitis* arthroconidia intrathecally; survival was followed and histologic analysis was performed. Mice lacking IL-6, IFN-g, IL-12p40, or iNOS exhibited survival and severity of meningitis that were essentially identical to those observed in wild type (WT) mice with the same genetic background (C57BL/6). In contrast, TNF-a knockout mice demonstrated significantly shorter survival ($p<0.05$) and a higher incidence of severe meningitis (70 % *vs.* 30%) and meningeal arteritis (70% *vs.* 40%) compared to WT mice (B6 129SF2/J). These results suggest that endogenous IL-6, IFN-g, IL-12p40, or iNOS may play less important roles than TNF-a in the immune response of the CNS to *C. immitis* infection in our murine model. It remains to be determined whether differences in the production of these IMM can affect the severity of pathological processes that underlie the clinical manifestations of the infection.

6. Branching Hyphae In Cerebrospinal Fluid Of Four Patients With Coccidioidal Meningitis And Review Of The Literature

R. Al-Kuran, R. Johnson, C. Younan, R. Singh, H. Einstein

University of California at Los Angeles, Kern Medical Center, Bakersfield, California

Coccidioidal meningitis is a frequent presentation of disseminated cocci, presenting in as many as 1/3 to 1/2 of all patients with dissemination. It is infrequently diagnosed by observing the organism in the cerebrospinal fluid in its parasitic phase (spherules and endospores). Even more rare is the finding of mycelia, the saprophytic form, in the CSF of infected individuals.

We report four cases of coccidioidal meningitis where the hyphal form of the organism was recovered from the cerebrospinal fluid. All four patients had ventriculoperitoneal shunts placed for hydrocephalus, a frequent complication of this devastating disease. One patient represents the first female observed having the hyphal forms in the CSF. Our second patient is the first reported pediatric case with such findings.

Review of the literature reveals only 10 cases of the mycelial forms involving the CSF or CNS tissue since the 1900's. With the exception of one case with *Coccidioides immitis* in a CNS granuloma, all were males, ages ranging from 26-56. Of the cases where the races were mentioned, 3 white, 2 Latin, and 2 Asian patients were reported. Two of the reported cases had associated immune suppression due to HIV infection, and another with renal homograft and insulin dependent diabetes mellitus for more than 30 years. With the exception of 2 cases, all of them had intraventricular shunt devices or reservoirs placed for relief of hydrocephalus.

To date, the cause and mechanism leading to this morphological reversion in vivo has been speculative. Treatment beyond removal/replacement of shunts in those cases involving such devices has not differed from the treatment of coccidioidal meningitis in general.

We hope our reported cases will increase the awareness of the variation of the anticipated biological findings and facilitate diagnosis.

7. Coccidioidomycosis in the Transplant Programs at Mayo Clinic Scottsdale

Janis E. Blair, MD

Mayo Clinic Scottsdale, Scottsdale, Arizona

Introduction: Coccidioidomycosis (cocci) is an endemic fungal infection of the southwestern USA. Prior studies from transplant programs located in endemic areas showed that coccidioidal infections occur in 4-9% of patients. Risk factors for post transplantation coccidioidomycosis included a pretransplant history of cocci, a positive serology, or treatment for rejection. In this group of patients, coccidioidal infection is frequently extrapulmonary and has significant mortality.

Aim: To review the incidence of coccidioidomycosis in patients who have received liver, kidney and bone marrow transplants at our institution, and assess for any additional risk factors for infection.

Methods: A retrospective chart review was performed on all patients undergoing transplantation at our institution. Patients were identified using transplant program databases. Kidney and liver programs were reviewed since the onset of the program in June 1999 through December 2002. All kidney and liver transplant recipients had serology performed (enzyme immunoassay, complement fixation and immunodiffusion) at the time of a pretransplant evaluation, repeated at transplant, and periodically thereafter. All patients with a positive history or serology underwent chronic azole suppression, the duration lasting 6-12 months or longer according to a specified protocol. All kidney recipients received 1 month of fluconazole for candida prophylaxis. Liver recipients received 30-day antifungal prophylaxis if deemed high risk according to predetermined criteria. Within the bone marrow program, patient charts were reviewed from August 1993 through January 2003. During this time, antifungal strategies evolved. Current patients are screened with serology (complement fixation and immunodiffusion). All patients receive fluconazole 400 mg/daily from the beginning of conditioning regimen through marrow engraftment.

Results: 124 patients received liver transplants. 6 patients received fluconazole for positive history or serology. One patient with a history of remote cocci did not receive antifungal prophylaxis. None of these 7 patients had recurrent coccidioidomycosis. 2 patients (1.6%) had symptomatic coccidioidomycosis at 4 months post transplantation, 1 had extrapulmonary infection, and both survived. 134 patients underwent kidney transplantation. 6 kidney recipients received chronic azoles for positive history or serology; none had post transplantation coccidioidal infection. Three (2%) kidney recipients contracted cocci infection, 2 had disseminated infection and 1 died. 123 patients underwent bone marrow transplantation (13 allogeneic and 110 allogeneic). One (1.6% of total bone marrow recipients) allogeneic bone marrow recipient had fatal, disseminated coccidioidomycosis. No autologous bone marrow recipients had documented coccidioidomycosis following transplantation.

Conclusions: Targeted antifungal prophylaxis in the liver and kidney transplant programs resulted in a low incidence of coccidioidomycosis. Routine antifungal prophylaxis in the bone marrow recipients also resulted in a low incidence. Of 6 post-transplant coccidioidal infections, extrapulmonary (66%) and fatal (33%) infections were prominent. With current antifungal strategies, coccidioidomycosis is an infrequent but serious infection in transplant recipients residing in an endemic area.

8. Voriconazole treatment for refractory Coccidioidomycosis

Rodney D. Adam, Leonard Ditmanson, Deborah Goldsmith, and John Galgiani

University of Arizona College of Medicine and Valley Fever Center for Excellence

There is an urgent need for new agents for treatment of coccidioidomycosis, and especially for disseminated disease. The currently available agents are limited by toxicity (especially the amphotericin compounds) and by suboptimal efficacy (amphotericin and azoles). Voriconazole is a new azole with very good oral absorption and good CNS penetration as well as excellent in vitro activity for Coccidioides immitis. However, animal studies have not been performed because of its short half life in the mouse model and human studies have also not been done. Therefore the current series of patients consists of seven cases with disseminated

coccidioidomycosis in which therapy with other drugs had already failed. A total of seven patients have been treated with voriconazole at daily doses of 400 mg to 600 mg. Five of the seven are immunocompromised (four HIV and one steroid-treated) and five have meningitis as the major site of dissemination. The drug has been well tolerated although one patient developed visual symptoms which prevented the dose from being increased beyond 400 mg/day. Five have been followed for four to seven months and symptoms and signs have improved in all. The other two have been treated for only two months, so the followup is inadequate to assess the response. These observations indicated that voriconazole is a promising addition to the antifungal repertoire for treatment of coccidioidomycosis. However, a longer period of followup is needed to determine more adequately the efficacy as well as toxicity of voriconazole. Ideally, prospective studies should be performed in patients with newly diagnosed disseminated coccidioidomycosis to allow a more accurate comparison with other agents.

9.Serologic Diagnosis of Coccidioidomycosis using Commerically Prepared Reagents: Discrepancies between EIA Screening and Immunodifficusion Confirmatory Studies.

Jestila, CM

Abstract not Submitted

10. Immunohistochemical Staining for Lymphocyte Subset and Cytokines in Human Coccidioidal Granulomata

Lijin Li, Margaret A. Rennels, and Neil M. Ampel
University of Arizona and Southern Arizona Veterans Health Care System

The in situ immunologic response in human coccidioidomycosis remains undefined. To explore this further, pulmonary necrotizing coccidioidal granulomata were examined using immunohistochemical staining for lymphocyte subsets and for the cytokines interleukin-10 (IL-10) and interferon-? (IFN-?). Discrete perigranulomatous lymphocytic clusters were seen in 8 of 9 tissues examined. In these tissues, T lymphocytes (CD3+) significantly outnumbered B lymphocytes (CD20+) in the mantle area of the granulomata ($p = 0.028$), whereas the clusters were composed of roughly equal numbers of T and B lymphocytes. While the number of cells in the mantle expressing IL-10 was similar to those in the perigranulomatous clusters, there were significantly more cells expressing IFN-? in the mantle compared to the clusters ($p = 0.037$). This is the first report noting perigranulomatous lymphocyte clusters and IL-10 in association with human coccidioidal granulomata. Both findings suggest that down-regulation of the cellular immune response is occurring within coccidioidal granulomata.

11. Susceptibility to Pulmonary *Coccidioides immitis* Infections in Inbred Mice is Due in Part IL-10

Joshua Fierer and Lory Walls.

Coccidioidomycosis is endemic in the southwestern US. The fungus is inhaled and initiates a pulmonary infection that either resolves spontaneously or disseminates hematogenously in genetically susceptible individuals. Little is known about the basis for genetic susceptibility to this infection, but inbred mice also differ in their susceptibility to *C. immitis*. We have previously shown that DBA/2 mice are resistant and B/c and B6 mice are susceptible to *C. immitis* peritonitis, and that resistance is dominant. We now compared pulmonary infections in DBA/2 and B6 mice and B6 mice had higher numbers of organisms in their lungs, were more likely

to disseminate to the spleen, and had a higher mortality. Cytokine mRNA levels were measured in the lungs and spleen of infected mice and IL-10 levels were at least 10-fold higher in B6 mice on days 10 and 16 after infection. B6 IL-10KO were more resistant than B6, and (DBA2xB/c.hIL-10+/- transgenic)F1 were more susceptible than DB/cF1. High levels of IL-10 in the transgenic mice down-regulated IFN? mRNA levels in vivo in infected lungs. Thus, high levels of IL-10 in infected mice are deleterious and explain in part the genetic susceptibility of B6 mice to this fungus infection. The role of IL-10 in human coccidioidomycosis remains to be established.

12. Further analysis of vaccine candidates to prevent Valley Fever: Protection of mice against intraperitoneal or intranasal Coccidioides infection by recombinant Coccidioides-specific antigen (CSA) and Antigen 2/PRA (Ag2/PRA), singly and in combination.

Yu J-J, Shubitz L, Peng T, Orsborn KO, Kirkland TN, Cole GT, Galgiani JN.

University of Arizona and SAVAHCS, Tucson AZ; VAMC and UCSD, La Jolla CA: and Med. Coll. Ohio, Toledo OH.

Ag2/PRA is a 196 amino acid (a.a.) proline-rich cell-wall protein which accummulates in maturing coccidioidal spherules and as a vaccine protection against intraperitoneal murine coccidioidal infection has localized to the N–terminal recombinant 1-106 a.a. fragment (PRA106). CSA is a 146 a.a. protein that is expressed in both saprobic and parasitic growth. We have now examined the immunogenicity of recombinant PRA106 expressed in Saccharomyces cerevisiae and CSA expressed in Eschericia coli.

Subcutaneous vaccination of C57B/6 mice with either antigen and CpG in MPL-oil as adjuvant (CpG/MPL) resulted in specific splenocyte stimulation in vitro as evidenced by lymphocyte blastogenesis and interferon-? secretion. 2 ?g of CSA, Ag2/PRA, or the combination administered intradermally with 10 ?g of CpG as an adjuvant reduced pulmonary cfu by more than 2.4 logs two weeks after intraperitoneal infection with Coccidioides arthroconidia. Whereas 0 of 20 unvaccinated C57B/6 mice survived beyond 21 days after 49 intranasal arthroconidia, mice (10 per group) receiving s.c. 1 ?g CSA, PRA106, or the combination with CpG/MPL increased survival at 60 days to 30%, 60%, and 90% ($p<0.0003$ for each compared to CpG/MPL alone). Percentage of mice with sterile spleens followed the same pattern (0%, 30%, 70%, and 80% respectively).

Multivalent recombinant vaccine candidates such as CSA plus the Ag2/PRA fragment may improve protection against coccidioidomycosis.

13.Mechanisms of Dendritic Cell-Mediated Immunity to Coccidioides immitis.

Douglas F. Lake, Neil Ampel

University of Arizona and SAVAHCS
Tucson AZ

Dendritic cells play a pivotal role in the immune response to *Coccidioides spp*. It is not known how antigen-specific immune tolerance in coccidioidomycosis occurs. The overall objective of our research is to understand the mechanisms of *C. immitis*-specific tolerance in humans mediated by dendritic cells (DC) with a long-term goal of identifying cell types, cell-surface receptors and fungal components that induce suppressive immune responses. We have shown that pulsing DC with *C. immitis* spherule lysate can reverse antigen-specific anergy in vitro. Paradoxically, when DC are generated in the presence of *C. immitis*

spherule lysate, they take on a phenotype that has been shown to suppress immune responses. Based on the problem of *C. immitis* -specific anergy in vivo and our data showing a suppressive DC phenotype when human monocytes are generated in the presence of *C. immitis* lysate, we hypothesize that suppressive antigenic components derived from *C. immitis* spherule lysate subvert cellular immunity and induce antigen-specific tolerance. We pulsed DC with T27K and showed induction of a DC2 phenotype that suppresses protective Th1 immunity. Specifically, CD1a expression was suppressed, while CD1d was upregulated on T27K-pulsed DC. CD1d is known to activate NK T cells, a suppressive lymphocyte population. NK T cells were detected when PBMC were incubated with T27K-pulsed DC, but not when PBMC were incubated with unpulsed DC or DC pulsed with a control antigen. These finding may provide insight into the specific mechanisms of Coccidioides-specific anergy.

14. Status of the Valley Fever Vaccine Project.

R.F. Hector and the Valley Fever Vaccine Project Investigators.

The Valley Fever Vaccine Project is in the 4th year of a 5-year effort to identify, evaluate and develop a suitable vaccine for the prevention or amelioration of coccidioidomycosis. Through the efforts of the five academic-based laboratories, a series of recombinant antigens have been identified and testing using murine models and associated immunologic assays. At present, three antigens are considered candidates for the final vaccine; each has been shown to increase survival and decrease fungal burden in a variety of mouse models. In addition, the Project is sponsoring efforts to establish a primate model of coccidioidomycosis, and is also conducting incidence/prevalence studies in naturally-acquired disease in domestic dogs. Efforts are also underway to establish a manufacturing process for the first antigen in a yeast vector, as well as establish analytical and formulation methods. The Project is also conducting a Phase 1 study of the skin test antigen coccidioidin in human volunteers as a prelude to anticipated incidence/prevalence studies in target populations in California and Arizona.

15. Disseminated Coccidioidomycosis in a Working Guide Dog

Davidson, Autumn P.

A 5 YEAR OLD NEUTERED MALE German Shepherd was presented for lethargy of two weeks duration. A generalized ulcerative dermatopathy unresponsive to cephalexin was diagnosed. The dog was referred to a veterinary dermatologist for further evaluation.

On examination, Cairo was lethargic and febrile (104.8 F), clinically dehydrated, had a stilted gain and generalized deep cutaneous ulcerations with suppurative crusts.

Intravenous fluids were initiated and diagnostic tests performed. ACBC showed lymphopenia, chemistry panel found hypoalbuminemia, and a urinalysis was normal. Skin scrapings were negative for parasites, skin cytology found pyogranulomatous inflammation with mixed bacteria.

A skin biopsy identified severe deep suppurative pyogranulomatous inflammation, and bacterial culture of the biopsy grew to species of Enterococcus. An antinuclear antibody test was positive at 1:640.

Treatment was initiated with enrofloxacin (136mg q24h) and prednisone (40 mg bid) pending diagnostics. Initial improvement in attitude, appetite and body temperature was noted. The prednisone was discontinued after a diagnosis of “German Shepherd Deep Pyoderma” was made. Differentials included erythema multiforme, toxic

epidermal necrolysis, pemphigus complex or systemic lupus erythematosis (SLE).

Fever, limb edema, mucocutaneous ulceration and generalized bruising occurred within 48 hours. Additional diagnostics performed included tick borne disease serologies (Rocky Mt. Spotted fever, Ehrlichia canis, Lyme). Serologies were negative. Cairo was placed on doxycycline (200mg bid) and prednisone restarted at 60mg q24h, and was referred to Guide Dogs for the Blind, Inc. for further evaluation.

A repeat hemogram identified thrombocytopenia and a mature neutrophilic leukocytosis. Hypoaalbuminemia was still evident. The liver enzymes were elevated compatible with corticosteroid use. Proteinuria was identified in a urinalysis, the protein creatinine ratio was significant at 6.5. Thoracic radiographs showed mild pleural thickening, and abdominal ultrasound found hepatomegaly with homogenous parenchyma. A repeat antinuclear antibody test was positive at 1:3200. A coagulation profile was normal except for hyperfibrinogenemia. A repeat skin biopsy identified suppurative dermatitis and vasculitis. Arthrocentesis was suppurative.

Cairo's problem list included thrombocytopenia, dermatisit, vasculitis and polyarthritis. The tentative diagnosis was systemic lupus erythematosis. Prednisone was continued at 2.2mg/kg/day, enrofloxacin at 136mg po q 24h, and azathioprine at 50 mg/M^2.

Initial improvement again occurred, followed by acute dyspnea and collapse 5 days later. Thoracic radiographs identified a diffuse mixed small nodule pattern with perihilar consolidation and pleural effusion. Blood cultures were negative for bacterial growth.

Differentials included pulmonary hemorrhage, ARDS, and granulomatous lung disease. Cairo was euthanized due to his moribund condition.

Post mortem examination found pyogranulomatous lymphadenitis, splenitis and hepatitis. A pyogranulomatous pneumonia was identified. Alveoli frequently contained numerous neutrophils and macrophages with large yeast-like structures that had thick basophilic outer walls and multiple internal endosproes. The organism was roughly 30-40 microns in diameter and was adjointed by granulomatous and neutrophilic inflammation. Coccidioidomycosis serology was performed on an ante mortem serum sample. The precipitin test was negative, the quantitative immunodiffusion test positive at 1:16.

The diagnosis was disseminated coccidioidomycosis. Cairo was born in San Rafael, California, but lived in the San Joaquin valley from 2 to 12 months of age. He then returned to San Rafael for Guide Dog training, graduating when 2 years of age. He worked in Stone Mt. Georgia between the ages of 2 and 5 years, with no travel history into an area endemic for coccidioidomycosis. His puppy and training dog medical histories were unremarkable.

This case of disseminated coccidioidomycosis likely represents recrudescence of a previously occult, asymptomatic pulmonary infection contracted 4 years previously, included by pharmacological immunosuppression. No evidence of cutaneous coccidioidomycosis could be demonstrated. The development of lifelong immunity following recovery from coccidioidal infection has been surmised but never proven in the dog. Immunosuppressive therapy in dogs originating from areas endemic for coccidioidomycosis should be undertaken with caution.

16. Update on Epidemiology of Canine Coccidioidomycosis

Shubitz, Lisa F.

Department of Veterinary Science and Microbiology
University of Arizona, Tucson, AZ

Background: Dogs suffer clinically from Valley Fever and it is felt canines would benefit from availability of a vaccine to prevent it. Little research has been performed to document the number of cases of canine coccidioidomycosis. The purpose of the study was to determine the incidence of Coccidoides exposure in dogs, evaluating both asymptomatic infection and clinical disease. A prospective study was designed beginning with seronegative puppies and screening and evaluating health for one year. Due to slow enrollment, a second design was instituted to study a cross-section of dogs restricted to overlap with the prospective puppies by age. Incidence and prevalence, respectively, of Coccidioides infection was determined for the studies.

Methods: 4-6 month old puppies were screened for general health and determined to be AGID seronegative for Coccidioides at initial testing. Enrolled puppies were tested at 6-month intervals for a 1 year period. Interested owners were encouraged to come back at additional 6-month intervals for testing. Puppies becoming ill during the study period were worked up with serum chemistries, complete blood counts, and coccidioidal serology testing. Only 124 dogs were able to be recruited for the prospective study. In order to try to expand the data base and fulfill the original goal of enrolling 400 dogs in a reasonable time frame, a prevalence study with dogs of overlapping ages was felt to be the best substitute. We recruited owners to participate by submitting a single serum sample from any dog, sick or healthy, between 4 and 18 months of age. In this modified protocol, we enrolled 365 dogs in 1 year.

Results: Of 124 prospective dogs, 25 (20%) tested seropositive for Coccidioides at least once during the study; of the 25 dogs, 20 (16%) were asymptomatic and 5 (4%) were ill with coccidioidomycosis. Among prevalence study dogs selected only for age of dog, 32/365 (9%) of dogs were seropositive and 19(5%) were asymptomatic while 13(4%) were clinically ill. Because of the few numbers of actually ill dogs, the two studies were combined to estimate rate of clinical disease. Using a Weibull survival analysis, the incidence of disease in dogs during the first year of life was 2% and
the incidence during the second year of life was 4.3%, with a cumulative incidence 6.3% in the first two years of life.

Conclusions: The study is incomplete and the data not all in yet, however in an earlier interim analysis of the data, the two studies were much more similar in asymptomatic exposures. As the study has progressed, an increasing difference in age of the dogs at testing seems to be separating the results. The likelihood of exposure is higher in the prospective than prevalence dogs. Average age of last test in the prospective dogs was 442 days (122-720) while average age of prevalence dogs was 277 days (120-540). Interim conclusions are that asymptomatic coccidioidomycosis is a relatively frequent occurrence in the dog, and that likelihood of exposure increases with increasing age.

Annual Presentations of Interesting Coccidioidomycosis Cases

Disseminated Coccidioidomycosis After Corticosteroid Therapy

Rafael Laniado-Laborin MD, MPH, FCCP and
Noemí Cabrales-Vargas MD

Hospital General de Tijuana, ISESALUD, México

Extrapulmonary dissemination occurs in 4-5% of clinical apparent cases of

coccidioidomycosis, specially in patients with immunosuppression.

Clinical Case

The patient is a 36 year-old Mexican male, with no significant past medical history. The patient was first seen by a physician after 1 month of flu-like symptoms, mild fever, myalgias and dyspnea. At that time, the physical examination was normal, and the chest x-ray showed a discrete, barely perceptible air-space opacity at the right lung base. A diagnosis of bronchial asthma was established and the patient received treatment with claritromycin and two doses (a week apart) of IM long acting (approximately three weeks) betamethasone, with an effect equivalent to a 40 mg/day of prednisone.

Two weeks later the patient developed high fever, productive cough of purulent sputum, multiple vesicular skin lesions and abdominal distension. He was referred to our hospital and admitted 6 January 2003 (two months after the appearance of the first symptoms). His vital signs were 60.8 kg, BP 110/60, P 140 x', T: 38.6°C and R: 28 ´'. There was a small abscess in left eyebrow region, moderate respiratory distress, and abdominal distension with an abdominal perimeter of 102 cm (40 in) and hepatomegaly.

Chest x-rays now showed an air-space opacity in the right lung base and upper mediastinal widening. Abdominal ultrasound confirmed the presence of ascites and hepatomegaly.

Routine laboratory studies demonstrated a leukocyte count of 17,500/ mm3 (78% PMN), ALT 50 U/L (4-44 U/L), alkaline phosphatase 150 U/L (30-140) and gamma GT 83 U/L (7-52 U/L). The HIV test was negative. An abdominal paracentesis revealed exudative serofibrinous fluid; smears and cultures were later reported as negative for AFB and fungi. Smears from the facial abscess showed abundant *Coccidioides immitis* spherules; culture was later reported as positive for C. immitis. Serology was positive with a CF of 1:64.

Treatment was started with intravenous fluconazole 1,000 mg/day. After two weeks treatment was changed to oral route with itraconazole (800 mg/day) for a month. Currently he is receiving 600 mg of itraconazole/day. The patient is asymptomatic, his chest x-ray is now normal and the ascites fluid was totally reabsorbed.

Conclusions

Several reports have described disseminated coccidioidomycosis during chronic corticosteroid therapy. The only risk factor for dissemination in this case was the use of systemic corticosteroids. Nonetheless, we have to acknowledge that although we could not demonstrate immunosuppression, there was no definite proof of immunocompetency. This case is a good reminder that systemic steroids should not be used liberally in a patient without definite diagnosis in a coccidioidomycosis endemic area.

"A Case of Coccidioidomycosis In The South of México"

Hernández M L, Zavala P M, Rivera B C, Hidalgo L
H.Torres SnM G P

A 29 years old male had the diagnosis of osteomyelitis of the pelvic limb. He was married, blue collar worker, born in the State of México, resident of Tijuana, Baja California, México. Family record of diabetes mellitus II. Smoker of 1 package a year. Alcoholism positive, used drugs as marijuana, cocaine and amphetamines since he was 7 years old until 26 years old. He had 3 heterosexual couples. His illness had one year evolution characterized by the appearance of blebs on the posterior face of the thorax that disseminate quickly until they covered all the posterior surface of the thorax, itching, confluents, measured 1 cm X 1 cm, spreading in one week to the abdominal wall and cervical surface, reaching the left periauricular area. Posteriorly the blebs converted into "costras melicéricas" drying completely, leaving hypochromic maculae. Only one of these lesions was

appreciated with irregular borders which ulcerated and extended to the cervical region =and posterior face of the thorax.

Five moths previously to his hospitalization, presented a small bleb of one centimeter of diameter which grew and ulcerated with frank purulent secretion in a moderate to abundant amount. 1 month previously to his hospitalization suffered spontaneous fracture-dislocation of the ankle as well as night fever occasionally. He presented to the hospital because of dissemination of the skin lesions to the anterior and posterior faces of the thorax, ears and occipital area, difficulty to walk due to pain in the right ankle and daily fever. At the physical examination he presented blood pressure 110/70 mmHg, heart rate 110x´, respiratory rate 20x´, temperature 37.5 degrees Centigrade. Alert, oriented in time, place and person, pale whit disseminated skin lesions that affected the head in the occipital area, neck, posterior face of the thorax and the proximal regions of the upper limbs, characterized by plaques with ulcers in some lesions and others with rubber like aspect. Normal skull, normal papillary light reflexes and isocoric pupils. Normal funduscopic examination, pale conjunctives +++, free patency of each nostril, oral cavity well hydrated, presented tooth cavities degree II on the upper molar. Simetric neck without adenopaties.
The point of maximal impulse was detected in the fifth intercostal space, midclavicular line. Respiratory sounds with no alterations, normoactive bowel sounds and no masses or tumors in the abdomen. Genitalia without alterations. Right pelvic limb with soft edema ++/++++, painful, located on the foot to he proximal third of the leg. He presented ulcers of 2X3 cm on the ankles and the anterior face of the foot with purulent secretion. Movility arc diminished about80% in the tibiofibulotarsal joint with normal pulse and normal deep tendon reflexes. Neurologically preserved. A mortise radiograph of the ankle showed osteopenic on the tarsal area and the distal extremities of the bones, such as the fracture-dislocation of the right ankle. He received initial treatment with amphotericin B. He was HIV negative detected with ELISA and western blot tests.

Cultures of secretions of the skin of the right ankle resulted Coccidioides immitis. The dermopathologic study showed irregular acantosis with enlargement and growing of the interpapillary processes. On the superficial dermis, medial and deep it was identified abundant linfohistiocitary infiltrate with giant multinucleate cells and some plasmocytes. There were several spherulae of thick wall with endospores that were phagocyted and had different sizes and were in different places of the dermis. The stroma was fibrous with areas of edema and eritrocyte extravasation. There were numerous dilated capillaries and congestioned with fibrin deposits. Grocot, ZN and PAS smears were made. It was concluded Coccidioidomycosis by cytopathology. He was treated with amphotericin B 1.5 grams total and continued with itraconazole 200 mgrs every 12 hours and showed clinical improvement. His follow up is done by infectology. The culture of purulent material resulted Staphyllococcus aureus and received treatment with cephalotine intravenously with culture negative after follow up.

The 2003—CSG47 Most Unusual Clinical Case 0f Cocci Award Winner

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Coccidioidal Endocarditis

Matt Hall, MD and Tsin Yeo, MD.
LDS Hospital, Salt Lake City, Utah

A 53-year-old man was referred to our institution for surgical management of infective endocarditis (IE). Eight

weeks prior he had transient left sided paresis and was found to have vegetation on his prosthetic aortic valve. Prior to presenting with the neurologic event he had three weeks of subjective fevers and malaise. Blood cultures were negative and treatment with vancomycin, gentamicin, and rifampin was initiated. However, his fevers persisted with the development of night sweats and headaches. Five weeks into therapy he had an embolic right renal infarct. Cetriaxone was added to his treatment regime. After three additional weeks of treatment without improvement, he was transferred to our institution for valve replacement. Two days prior to transfer amphotericin had been added. Cultures throughout the illness had been negative other than isolation of different species of coagulase negative staphylococci on two occasions. His prior medical history included an aortic valve replacement (bioprosthetic) two and half years ago for aortic stenosis related to a calcified bicuspid valve. Eight months prior to presenting with IE he had a pericardectomy for constrictive pericarditis. This hospitalization was prolonged by bacteremia from methecillin sensitive Staphylococcus aureus thought to have originated from an intravascular catheter. There was no valvular vegetation at that time. Five months ago he was treated for pulmonary coccidioidomycosis with two months of oral fluconazole. Initial evaluation on arrival to our institution he was found to be chronic ill appearing, but afebrile and hemodynamically stable. He did complain of headaches, nausea, and feeling extremely weak. His cardiac examination detected a soft systolic murmur heard at the upper sternal borders. A transesophageal echocardiogram (TEE) detected only a small, 1-2 mm aortic valve nodule with a normally functioning valve. Due to the unusual history, lack of microbiology evidence for IE, and the relative benign appearing TEE, watchful observation of the patient off all therapy was started.

After two to three days the patient felt better, but continued to have headaches and had developed low-grade fevers of 38.0 to 38.3. Multiple blood cultures were negative. However, on hospital day 12 the patient had an episode of aphasia lasting two hours. A CT scan of the head was negative. A follow up transthoracic echocardiogram found the aortic valve lesion to be up to 4-5 mm in size. The patient underwent an aortic valve replacement. A diagnosis was made from the resected valve. Histopathology of the vegetation found septate hyphae and rare spherules. White colonies grew on routine fungal media which after one week were found to be consistent with Coccidioides immitis on touch preparation with cotton phenol blue. PCR probe testing was also performed and confirmed the microscopic diagnosis. Further testing found the patient to have a Coccidioides IgG level of 5 and IgM of 1 by ELISA. A titer for Coccidioides complement fixation testing was 1:32. CSF of serology was also positive. A MRI of the head found evidence of basilar vasculitis secondary to meningeal inflammation. A final diagnosis of pulmonary Coccidioidomycosis with dissemination to include a prosthetic cardiac valve and meningitis was made.

List of Annual Meetings

(1)	07/18/56	San Francisco	
(2)	21/5-6/57	Los Angeles	
(3)	12/4-5/58	Los Angeles	
(4)	12/3-4/59	Los Angeles	
(5)	12/8-9/60	Los Angeles	
(6)	11/30 -12/1/61	Los Angeles	
(7)	11/29 - 30/62	Los Angeles	
(8)	12/5-6/6 3	Los Angeles	
(9)	12/10-11/64	Los Angeles	CTS
(10)	12/07/65	Phoenix	2nd Coccidioidomycosis Conference
(11)	4/19/67	Palm Springs	CTS
(12)	5/01/68	Fresno	CTS
(13)	4/15/69	San Diego	CTS
(14)	4/01/70	San Francisco	CTS
(15)	4/06/73	Newport Beach	CTS
(16)	4/05/74	Sacramento	CTS
(17)	9/30/74	San Francisco	CCTG
(18)	4/02/75	San Diego	CTS
(19)	7/31/75	San Diego	CCTG
(20)	1/14 -15/76	San Diego	CCTG
(21)	4/07/76	Palo Alto	CTS
(22)	5/18/77	San Francisco	ALA
(23)	4/ 5/78	Beverly Hills	CTS
(24)	5/15/79	Las Vegas	ALA
(25)	4/11/80	Sacramento	CTS
(26)	3/28/81	San Francisco	CTS
(27)	5/15/82	Los Angeles	ALA
(28)	3/20/83	La Jolla	CTS
(29)	3/14-17/84	San Diego	4th Coccidioidomycosis Conference.
(30)	3/08/86	Santa Barbara	
(31)	4/04/87	Los Angeles	
(32)	4/09/88	Los Angeles	
(33)	4/08/89	San Jose	
(34)	4/07/90	Berkeley	
(35)	4/06/91	Tucson	
(36)	4/04/92	Fresno	
(37)	4/03/93	Tucson	
(38)	8/24-27/94	Stanford - 5th	Coccidioidomycosis “Centennial” Conference
(39)	4/01/95	Bakersfield	
(40)	3/30/96	Scottsdale	
(41)	3/05/97	San Diego	
(42)	4/04/98	Visalia	
(43)	3/20/99	Tijuana, BC, Mexico	
(44)	4/1/00	Berkeley	
(45)	3/31/2001	Tucson	
(46)	4/6/2002	Davis, CA	
(47)	4/3/2003	Scottsdale, AZ	

Conferences held in conjunction with:

Abbreviations:CTS = California Thoracic Society

CCTG = Coccidioidomycosis Cooperative Treatment Group

ALA = American Lung Association